Original Article

Serum Fibronectin Levels in Acute and Chronic Viral Hepatitis Patients

Ayse Erturk[1], Erkan Cure[2], Zulal Ozkurt[3], Emine Parlak[3], Medine Cumhur Cure[4]

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[1] *Department of Infectious Diseases, School of Medicine, Recep Tayyip Erdogan University, 53100 Rize, Turkey*

[2] *Department of Internal Medicine, School of Medicine, Recep Tayyip Erdogan University, 53100 Rize, Turkey*

[3] *Department of Infectious Diseases, School of Medicine, Ataturk University, 25240 Erzurum, Turkey*

[4] *Department of Biochemistry, School of Medicine, Recep Tayyip Erdogan University, 53100 Rize, Turkey*

Abstract

Background: **The aim of this study was to investigate the serum fibronectin (FN) levels and liver enzyme activities in patients with acute hepatitis (A, B, C) and chronic viral hepatitis (B, C); determine whether the virus types correlated with disease severity; and assess whether FN could be used as a marker of virus type or disease severity in patients.**

Methods: **A total of 60 subjects were enrolled in the study, including 20 patients with acute hepatitis (A, B, C), 20 with chronic hepatitis (B, C), and 20 healthy controls. Serum fibronectin (FN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), and albumin were measured in all patients from blood samples.**

Results: **Serum FN levels were significantly lower in acute (122.9 µg/mL (SD 43.1), $P < 0.001$) and chronic hepatitis patients (135.7 µg/mL (SD 46.0), $P < 0.001$) compared to controls 221.4 µg/mL (SD 32.5). A negative correlation was found between serum FN and AST ($r^2 = 0.528$, $P < 0.001$), ALT ($r^2 = 0.425$, $P < 0.001$), and GGT ($r^2 = 0.339$, $P < 0.001$). Additionally, high serum GGT levels ($\beta = -0.375$, $P = 0.010$), and low serum albumin levels ($\beta = -0.305$, $P = 0.008$) were associated with low serum FN levels.**

Conclusion: **Serum FN levels were lower in both acute and chronic hepatitis patients, and an inverse relationship between serum FN and serum AST, ALT, and GGT levels was found. A decrease in serum FN levels may indicate hepatitis severity as AST and ALT represent hepatocyte damage.**

Keywords: *fibronectin, hepatitis, chronic hepatitis, liver fibrosis, albumin*

Introduction

Viral hepatitis is associated with significant morbidity and mortality worldwide. Viral hepatitis generally refers to the five well-known hepatotropic viruses: hepatitis A, B, C, D, and E. It can also refer to other viruses, such as the hepatitis G virus (HGV), transfusion transmitted virus (TTV), TTV-like mini virus (TLMV), TTV variants (including SANBAN, TUS01, PMV, and YONBAN), SEN virus (SENV) subtypes (including SEN-V-D and SEN-V-H), Epstein-Barr Virus, cytomegalovirus, herpes simplex virus, varicella-zoster virus, and rubella. Hepatotropic viruses are further divided into enteral and parenteral groups based on their mode of transmission. Hepatitis A and E viruses are enterally transmitted and usually lead to self-limited acute hepatitis. Hepatitis B, C, and D viruses are parenterally transmitted, occur both in the acute and chronic forms, and, when they persist in a chronic carrier state, serve as a reservoir for infection and give rise to chronic hepatitis, cirrhosis, and hepatocellular carcinoma.

Acute hepatitis, liver cell necrosis, and inflammation of the liver are associated with an entity. The term “chronic hepatitis” is used to describe liver inflammation and necrosis in cases lasting more than six months because the clinical findings of chronic hepatitis are often silent (1–3). Diagnosis is generally made during routine blood screening based on moderate increases in

aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Serum bilirubin and albumin levels are generally normal, except in serious illness (4,5). Detection and monitoring of liver function abnormalities to assess various aspects of cellular integrity and structural damage are widely used and simple to perform. These include biochemical tests for aminotransferases (ALT, AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase (GGT), bilirubin, albumin/globulin rate, and prothrombin time (1,2,6).

Fibronectin (FN) is a high-molecular weight (~440 kDa) glycoprotein of the extracellular matrix (ECM) that binds to membrane-spanning receptor proteins called integrins (7). FN is synthesised by many cell types. A large portion of circulating FN is produced by hepatocytes, in which it exists in two forms, termed cellular FN (cFN) and plasma FN (pFN) (7–10). In healthy subjects, the human plasma FN level is ~ 300 ± 100 µg/mL (8), with no differences according to gender or age (11). pFN levels decrease over the course of acute and chronic hepatitis, and are associated with these levels of protease and activity, increased consumption of FN and the reduction of synthesis (10,12,13).

The aim of this study was to investigate the serum FN levels and liver enzyme activities in patients with acute hepatitis (A, B, C) and chronic viral hepatitis (B, C); determine whether the virus type correlated with disease severity; and assess whether FN could be used as a marker of virus type or disease severity in patients.

Materials and Methods

Patient selection

A total of 60 subjects was enrolled in the study: 20 patients (6 female, 14 male) with acute hepatitis (12 patients with hepatitis A, 4 patients with hepatitis B, 4 patients with hepatitis C); 20 patients (3 female, 17 male) with chronic hepatitis (16 patients with hepatitis B, 4 patients with hepatitis C); and 20 healthy controls (3 female, 17 male). The study groups were divided into subgroups according to the presence of viral hepatitis A, B or C; no other causes of viral hepatitis were included. Individuals were enrolled as patients from the Clinical Microbiology and Infectious Disease Clinics, Ataturk University Research Hospital over a one-year period following their granting of informed consent. The study conformed to the Helsinki Declaration and was approved by the local ethics committee of Ataturk University, Erzurum, Turkey.

Collection of samples

Approximately 10 mL of venous blood were taken from the subjects and healthy controls. Five millilitres of each blood sample were transferred to Vacutainer® tubes for an assay of AST, ALT, GGT, ALP, LDH, and albumin levels by a spectrophotometric method. The remaining 5 mL of blood was centrifuged at 3000 rpm for 5 minutes, and serum samples were transferred to Eppendorf tubes and stored at –80 °C for FN analysis. All laboratory studies were performed at Ataturk University, Medicine Faculty, and the Department of Biochemistry.

Analysis of fibronectin

Serum samples were thawed 12 hours before FN analysis. Human FN enzyme-linked immunosorbent assay (ELISA) kits (Bender Medsystem GmbH, Code No. BMS2028, Vienna, Austria) were used to analyse FN according to the manufacturer's protocol.

AST-to-platelet ratio index (APRI) score

The APRI score was calculated for cases of chronic hepatitis B and C based on formula (14): APRI = (AST level ULN/platelet counts (10/L) × 100, where upper limit of normal (ULN) represents the upper normal AST value or 55 IU/L.

Statistical analysis

The results were reported as means (SD). Data were analysed using the software package used for statistical analysis (SPSS) for Windows (version 13.1; SPSS, Chicago, IL, USA). The differences in FN levels and liver enzymes between the chronic hepatitis B or C subgroups and each binary group were determined using Mann-Whitney U-tests. Linear regression analysis was performed to determine the independent relationship between FN and albumin levels and other liver enzymes. The relationships among the variables were analysed by means of Spearman's correlation. Differences were considered significant at $P < 0.05$.

Results

The average age of patients was as follows: 31.80 years (SD 16.70) for the acute hepatitis group (30% females: 70% males); 39.7 years (SD 11.2) for the chronic hepatitis group (15% females: 85% males) and 35.4 years (SD 8.9) for the healthy control group (15% females: 85% males). The biochemical parameters of the acute hepatitis A, B, and C groups and the chronic

hepatitis B and C groups were divided into two groups, acute, and chronic, and each group was compared with the Mann-Whitney U test, which found that all of the biochemical values were similar for acute hepatitis groups and chronic hepatitis groups ($P > 0.05$). All the results for the acute hepatitis group are shown in table 1, while those for chronic hepatitis are shown in table 2. Regardless of whether the acute or chronic clinic was being considered, based on the etiologic agent, when the biochemical results were evaluated for patients with hepatitis A, B, and C virus infections, the FN level of the hepatitis A group was found to be lower than that of hepatitis B; however, this difference was not significant ($P > 0.05$), but the FN level of the A group was found to be significantly lower than that of the C group ($P = 0.020$). The albumin level of the hepatitis A group was found to be significantly higher than in the hepatitis B ($P < 0.001$) and C ($P = 0.002$) groups. All results are shown in table 3. Regardless of the etiologic agent, when comparing the biochemical results for patients with acute and chronic hepatitis infection and the control group, the FN was found to be significantly lower in the acute group ($P < 0.001$) and the chronic hepatitis group ($P < 0.001$) based on the control group. The albumin level in acute hepatitis is quite lower than that of the control group ($P < 0.001$). All results are shown in table 4.

Spearman correlation for all groups

Correlation analysis (Table 5) indicated negative correlations between AST ($r^2 = 0.528$, $P < 0.001$), ALT ($r^2 = 0.425$, $P < 0.001$), LDH

Table 1: Fibronectin, albumin, and liver enzymes found to be similar when biochemical parameters were compared for acute hepatitis patients according to the etiologic agent (A, B, C). The results of all parameters were non-significant

	Acute Hepatitis A ($n = 12$)	Acute Hepatitis B ($n = 4$)	Acute Hepatitis C ($n = 4$)
Fibronectin (µg/mL)	106.7 (SD 42.1)	126.0 (SD 48.1)	146.1 (SD 37.5)
AST (U/L)	1130.33 (SD 1131.46)	982.17 (SD552.08)	373.60 (SD 205.06)
ALT (U/L)	1713.33 (SD 11162.18)	1702.00 (SD 828.18)	736.00 (SD 371.40)
LDH (U/L)	1212.22 (SD 1934.98)	975.33 (SD 485.16)	485.60 (SD 115.27)
GGT (U/L)	140.11 (SD 43.67)	108.50 (SD 27.60)	112.40 (SD 24.47)
ALP (U/L)	141.33 (SD 24.52)	188.33 (SD 95.81)	177.80 (SD 39.33)
ALB (g/dL)	3.25 (SD 0.22)	3.23 (SD 0.42)	3.08 (SD 0.23)

Abbreviations: albumin = ALB; alkaline phosphatase = ALP; alanine aminotransferase = ALT; aspartate aminotransferase = AST; gamma-glutamyl transpeptidase = GGT; lactate dehydrogenase = LDH.

Table 2: Fibronectin, albumin and liver enzymes found to be similar for both groups when biochemical parameters were compared for chronic hepatitis patients according to the etiologic agent (B, C). The results of all parameters were non-significant

	Chronic Hepatitis B ($n = 16$)	Chronic Hepatitis C ($n = 4$)
Fibronectin (µg/mL)	129.5 (SD 49.2)	160.8 (SD 15.4)
AST (U/L)	81.81 (SD 61.84)	70.50 (SD 47.93)
ALT (U/L)	146.00 (SD 150.50)	108.25 (SD 96.83)
LDH (U/L)	434.37 (SD 71.81)	432.35 (SD 67.71)
GGT (U/L)	58.19 (SD 32.61)	56.50 (SD 20.57)
ALP (U/L)	110.56 (SD 45.30)	112.00 (SD 33.66)
ALB (g/dL)	4.05 (SD 0.35)	3.75 (SD 0.23)

Abbreviations: albumin = ALB; alkaline phosphatase = ALP; alanine aminotransferase = ALT; aspartate aminotransferase = AST; gamma-glutamyl transpeptidase = GGT; lactate dehydrogenase = LDH.

Table 3: Regardless of acute and chronic status, in comparing the biochemical parameters of hepatitis A, B, and C, the fibronectin level was found to be lower in the hepatitis A group than the hepatitis B group, though not significantly lower – but significantly lower than in the C group. The albumin level in the hepatitis A group was significantly lower than that of the hepatitis B and C group. ALT and GGT values in the hepatitis A group were found significantly more than in the B and C groups

	Hepatitis A (n = 12)	Hepatitis B (n = 20)	Hepatitis C (n = 8)
Fibronectin (μg/mL)	106.7 (SD 42.1)	126.6 (SD 44.5)	135.2 (SD 40.5)
			P = 0.020¶
AST (U/L)	1130.33 (SD 1131.46)	777.06 (SD 1030.45)	449.50 (SD 574.84)
ALT (U/L)	1713.33 (SD 11162.18)	1225.44 (SD 1047.17)	815.80 (SD 1006.80)
		P = 0.004¶	P = 0.031¶
LDH (U/L)	1212.22 (SD1934.98)	944.55 (SD 1397.39)	537.90 (SD 220.20)
GGT (U/L)	140.11 (SD 43.67)	111.94 (SD 47.15)	97.50 (SD 45.74)
		P < 0.001¶	P = 0.007¶
ALP (U/L)	141.33 (SD 24.52)	168.83 (SD 62.13)	115.70 (SD 32.78)
ALB (g/dL)	3.25 (SD 0.22)	3.31 (SD 0.49)	3.66 (SD 0.43)
		P < 0.001¶	P = 0.002¶

Abbreviations: albumin = ALB; alkaline phosphatase = ALP; alanine aminotransferase = ALT; aspartate aminotransferase = AST; gamma-glutamyl transpeptidase = GGT; lactate dehydrogenase = LDH.
¶ P < 0.05 versus Hepatitis A group for Mann Whitney U test.

Table 4: Regardless of the etiologic agent (A, B, C) and chronic (B, C) hepatitis, when comparing the control group and biochemical parameters, fibronectin values were found to be low in the acute and chronic hepatitis groups based on the control group. The albumin level of the acute hepatitis group was found to be lower than that of the control and chronic hepatitis groups

	Control (n = 20)	Acute Viral Hepatitis (A, B, and C) (n = 20)	Chronic Viral Hepatitis (B and C) (n = 20)
Fibronectin (μg/mL)	221.4 (SD 32.5)	122.9 (SD 43.1)	135.7 (SD 46.0)
		P < 0.001¶	P < 0.001¶
AST (U/L)	28.05 (SD 13.77)	896.70 (SD 967.30)	79.55 (SD 58.34)
		P < 0.001¶	P < 0.001¶, P = 0.014$^{\pi}$
ALT (U/L)	23.50 (SD 12.01)	1465.60 (SD 982.36)	138.45 (SD 140.01)
		P < 0.001¶	P = 0.006 ¶, P < 0.001$^{\pi}$
LDH (U/L)	230.65 (SD 128.70)	959.50 (SD 1315.55)	434.05 (SD 69.24)
			P < 0.001¶
GGT (U/L)	29.80 (SD 35.98)	123.70 (SD 36.93)	57.85 (SD 30.11)
		P < 0.001¶	P < 0.001¶$^{\pi}$
ALP (U/L)	71.85 (SD 17.35)	164.55 (SD 58.95)	110.85 (SD 42.42)
		P < 0.001¶	P = 0.003 ¶, P = 0.007$^{\pi}$
ALB (g/dL)	3.76 (SD 0.47)	3.20 (SD 0.29)	4.00 (SD 0.35)
		P < 0.001¶	P = 0.018¶, P < 0.001$^{\pi}$
APRI score			0.90 (SD 1.2)

Abbreviations: albumin = ALB; alkaline phosphatase = ALP; alanine aminotransferase = ALT; aspartate aminotransferase = AST; gamma-glutamyl transpeptidase = GGT; lactate dehydrogenase = LDH; APRI = AST-to-platelet ratio index.
¶ P < 0.05 versus control group for Mann Whitney U test.
$^{\pi}$ P < 0.05 versus acute viral hepatitis for Mann Whitney U test.

(r^2 = 0.121, P = 0.006), GGT (r^2 = 0.339, P < 0.001) and ALP (r^2 = 0.301, P < 0.001) with FN. There was also a negative correlation between ALT (r^2 = 0.096, P < 0.016) and GGT (r^2 = 0.274, P < 0.001) with albumin. Furthermore, a significant negative correlation between the APRI score and FN was identified (r^2 = 0.275, P = 0.017), and although not significant, the APRI score and albumin were negatively correlated (r^2 = 0.275, P = 0.402).

Linear regression analysis

A linear regression analysis was performed, in which FN was used as the dependent variable and AST, ALT, LDH, GGT, ALP, and albumin were utilised as independent variables (Table 6). We found that GGT (β = –0.375, P = 0.010) and albumin (β = -0.305, P = 0.008) were independently associated with decreased FN.

Discussion

The treatment and monitoring of viral hepatitis has been widely studied, as the disease threatens the lives of thousands of people worldwide. This study compared biochemical liver function tests, and serum FN and albumin in patients with acute and chronic hepatitis. Serum FN and albumin levels were similar in all three acute viral hepatitis groups (A, B, C) and in chronic hepatitis (B, C). However, regardless of

Table 5: Positive correlation values defined as 'r', negative correlation values defined as 'r^2'

	FN	AST	ALT	LDH	GGT	ALP
FN	–	r^2 = 0.528 P < 0.001	r^2 = 0.425 P < 0.001	r^2 = 0.121 P = 0.006	r^2 = 0.339 P < 0.001	r^2 = 0.301 P < 0.001
AST	–	–	r = 0.566 P < 0.001	r = 0.484 P < 0.001	r = 0.518 P < 0.001	r = 0.545
ALT	–	–	–	r = 0.334 P = 0.009	r = 0.542 P < 0.001	r = 0.493 P < 0.001
LDH	–	–	–	–	NS	r = 0.275 P = 0.034
GGT	–	–	–	–	–	r = 0.545 P < 0.001
ALB	NS	NS	r^2 = 0.096 P = 0.016	NS	r^2 = 0.274 P < 0.001	NS

Abbreviations: albumin = ALB; alkaline phosphatase = ALP; alanine aminotransferase = ALT; aspartate aminotransferase = AST; gamma-glutamyl transpeptidase = GGT; lactate dehydrogenase = LDH; FN = fibronectin; NS = non-significant.

Table 6: Independent relationship between fibronectin and confounding variables by linear regression analysis (r^2 = 0.538, P < 0.001). GGT and albumin were independently associated with decreased fibronectin

Independent variables	Beta-regression coefficient	P
AST	–0.266	0.057
ALT	–0.790	0.614
GGT	–0.375	0.010
ALP	–0.196	0.080
LDH	–0.143	0.236
ALB	–0.305	0.008

Abbreviations: albumin = ALB; alkaline phosphatase = ALP; alanine aminotransferase = ALT; aspartate aminotransferase = AST; gamma-glutamyl transpeptidase = GGT; lactate dehydrogenase = LDH.

etiology, the FN levels of hepatitis patients were lower than those of controls. We found strong correlations between decreased serum FN, and GGT and albumin levels following univariate linear regression analysis. Similarly, there was a negative correlation between serum albumin and ALT and GGT levels.

The etiology of liver fibrosis remains unclear, and many hypotheses describing the relationship between FN and fibrosis have been proposed. A number of chronic events cause liver fibrosis (13), which is defined as the excess, disorganised accumulation of ECM components that causes the loss of normal liver cell functions (13,15,16). Although the standard procedure for the evaluation of liver fibrosis is hepatic biopsy (13), the invasive nature of the procedure, as well as the complication rate, sampling error and inter-observer variability, has led to the development of non-invasive methods. Currently available methods rely on two different approaches: a "biological" and a "physical" approach, thus there is a need to identify alternative evaluation markers (17–19). FN is an ECM non-collagen adhesive protein that plays a crucial role in intercellular adhesion, basal membrane adhesion, clot stabilisation, fibroblast migration and macrophage functions (12,20). The plasma form of FN is produced by hepatocytes and blood vessel endothelium and is soluble in blood and other body fluids. Insoluble FN is located in the ECM of macrophages, fibroblasts, and the surface of endothelial cells (12).

FN is significant in the development of early liver fibrosis and may act as a chemotactic factor for collagen-producing cells and as a skeleton for new collagen formation. Myofibroblasts are the primary cell type involved in physiological wound healing and its pathological counterpart, fibrosis. The FN splice variant extra domain A is hypothesised to mediate the differentiation of myofibroblasts. FN is among the first ECM proteins to be upregulated after injury. A previous study reported that FN, which is secreted early in the process of liver injury and is implicated in liver endothelial cell angiogenesis as well as hepatic stellate cell activation, might play a key role in mediating cross talk between these cell types in response to toll-like receptor activation (20).

Recently, there has been increased interest in detecting liver fibrosis through the application of non-invasive techniques. The APRI score is one of the most useful scores utilised to predict fibrosis (14,21). In our study, a negative correlation between APRI, and serum FN and albumin levels was found. However, the APRI scores of our patients were low, indicating a less severe level of liver fibrosis. In acute and chronic hepatitis, a decrease in serum FN levels is expected because FN is released from hepatocytes. A FN discriminant score based on FN, APRI, and albumin has been reported to predict liver fibrosis with a high degree of accuracy, potentially decreasing the number of liver biopsies required for monitoring hepatitis C (14,21,22).

Our data demonstrated a negative correlation between serum FN levels and AST, ALT and GGT levels. Serum FN levels decreased due to hepatocyte damage in patients with hepatitis and were lower in both acute and chronic hepatitis patients (23,24). Acharya et al. (23) reported that serum FN levels were lower in acute hepatitis patients than controls, and that this is a poor prognostic factor during fulminant disease that is related to an increased incidence of mortality. In the current study, serum FN levels decreased when ALT and AST increased. Since an increase in ALT indicates hepatocyte damage, decreased FN could be indicative of the severity of acute hepatitis.

Kandemir et al. (12) evaluated serum FN levels and liver enzymes AST and ALT in patients with chronic viral hepatitis B and C. They found that serum FN levels were significantly lower and AST and ALT levels were higher in patients than controls. They also found a negative, but non-significant, correlation between serum FN and, ALT and AST levels. Helvaci et al. (25) evaluated serum FN levels in chronic hepatitis B patients during the pre- and post-treatment periods. Therefore, FN might be a biochemical marker of treatment effectiveness in chronic hepatitis patients. However, one cannot conclude that the serum FN levels were not affected by disease etiology, since serum FN levels are less sensitive in evaluating disease.

In acute hepatitis, acute hepatocyte damage results in high AST and ALT levels. Nonetheless, serum FN levels in acute and chronic hepatitis patients were similar, and a strong correlation between FN and albumin levels was found in a linear regression analysis. This is not surprising since during inflammation, the level of albumin, as an acute-phase marker, decreases. With albumin levels, there is generally no decrease in acute hepatitis, while a decline is expected in chronic hepatitis, particularly in the presence of cirrhosis. Umemura et al. (26) reported that serum albumin levels were lower in acute than chronic hepatitis B patients, but this situation did not differ in both

acute and chronic hepatitis B fulminant cases. The lower albumin levels in acute hepatitis cases that we report here could be due to the inclusion of severe hepatitis cases. Because its half-life is approximately 20 days, albumin might decrease because the oral supply has failed in a one-month course of acute disease.

A previous study showed that the serum GGT level in hepatitis C was an independent predictor of significant fibrosis and proposed a non-invasive fibrosis test that included serum GGT (27). Other studies showed an independent association between higher serum GGT levels and the severity of fibrosis in both chronic hepatitis B and C patients (28,29). In the present study, we evaluated the factors associated with low serum FN levels in patients with acute and chronic hepatitis. We found that high serum GGT and low serum albumin levels were associated with low serum FN levels.

The results of this study support the use of FN as a predictor of disease and fibrosis severity because serum FN levels decreased in parallel with increased GGT levels. Lower serum FN levels in acute hepatitis have been reported; however, the relationship to GGT and the severity of acute hepatitis has not yet been defined. In our study, serum GGT levels were increased, while those of FN were decreased, which supports the use of FN as a marker of disease severity.

Limitation of This Study

Our study had several limitations. First, the limited number of subjects may not reflect the general population. Second, the causes of acute and chronic hepatitis were not included in the analysis. Third, the cross-sectional nature of the study did not allow for knowledge of serum FN levels in patients after the acute hepatitis infection had been resolved; serum FN levels might increase after eradication of chronic hepatitis. Finally, we did not include severe cirrhosis cases in this study; these cases should be considered for inclusion in future studies.

Conclusion

Serum FN levels were lower in patients with both acute and chronic hepatitis, and inverse relationships between serum FN and, serum AST, ALT, and GGT levels were found. A decrease in serum FN levels may be associated with hepatitis severity because AST and ALT are indicators of hepatocyte damage.

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Conflict of Interest

None.

Funds

None.

Authors' Contributions

Conception and design: AE
Analysis and interpretation of the data and obtaining of funding: EP, MC
Drafting of the article: AE, ZO
Critical revision of the article for the important intellectual content: EC, ZO
Final approval of the article: AE, EC
Provision of study materials or patient: ZO
Statistical expertise: EC
Administrative, technical or logistic support: AE, EP
Collection and assembly of data: MC

Correspondence

Dr Ayse Erturk
MD Infectious Diseases (Recep Tayyip Erdogan University)
Department of Infectious Diseases
School of Medicine
Recep Tayyip Erdogan University
53100 Rize, Turkey
Tel: +04 64213 0492/1719
Fax: +04 64217 0364
Email: ayseace25@hotmail.com

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